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RESEARCH ARTICLE
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Development and Evaluation of Nano-based Ocular Drug Delivery System for Glaucoma

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ABSTRACT

Brinzolamide (BZ) is an intraocular pressure-reducing agent with low solubility. The purpose of the present study was designed to increase the Brinzolamide bioavailability by Nano micellar formulation (NMs) as an ocular drug delivery system to increase the therapeutic efficacy. Brinzolamide Nano micellar were prepared by the solvent evaporation method by using a Rota evaporator. Based on initial release studies, different formulations were prepared in various ratios of Soya phosphatidylcholine, Polysorbate 80, and Sodium glycocholate. The prepared preparation was characterized by further physicochemical investigations such as drop size, Assay, pH, osmolality, Zeta potential, and viscosity. Brinzolamide micellar formulation (Trial-5) with suitable physicochemical properties exhibited high formulation stability under different conditions. These formulations included those with a low Drop Size of 28.8 µl, Osmolality 311 mOsmol/kg, Zeta potential -0.837 mV, pH 7.2 Drug content estimation (Assay) 101.9%, and stability for at least 6 months at 25 °C/40% RH and 40 °C/25% RH. The Brinzolamide ophthalmic without preservative solution was packed in a preservative-free container by using the Novelia packing system which enhanced the stability of the preparation throughout the in-use shelf life of the solution. Finally, it was concluded that Brinzolamide NMs without preservatives packed in the Novelia packing system will be an effective package model for the Brinzolamide ophthalmic solution.

Keywords: *Brinzolamide, Soya phosphotidyl choline, Boric acid, and Purified Water*

INTRODUCTION

The eye is probably the most important sensory organ of the body. It is responsible for sight and also visual cues for maintaining balance. The eye is also an immune-privileged site and is derived from the forebrain during embryological development, it retains the challenges of the blood–brain barrier for drug delivery in the form of the blood–retina barrier among others2. The anatomy and structure of the eye are very complex, and it is divided into two parts, i.e., the Anterior segment and the posterior segment of the eye; the portion front to the lens is called an anterior segment, whereas the portion behind the lens of the eye is referred to as the posterior

segment. The anterior segment of the eye consists of the cornea, pupil, iris, ciliary body, conjunctiva, anterior chamber, lens, and aqueous humor; moreover, the posterior segment of the eye contains vitreous humor, sclera, choroid, and retina1.

The diseases of the anterior segment of the human eye generally include corneal infections and disorders like pterygium, Fuchs' dystrophy, dry eyes, corneal neovascularization, and autoimmune disorders, e.g., cataracts. However, other types of diseases are referred to as disorders of the posterior segment such as glaucoma, cytomegalovirus retinitis, age-related macular degeneration, Diabetic retinopathy, Retinitis pigmentosa (RP), Proliferative vitreoretinopathy and Uveitis1. For managing diseases in the anterior segment of the eye, topical administration is the most common route. Drug transport via corneal/non-corneal routes involves several intricate biological processes; consequently, the bioavailability of topically applied drugs is poor in the internal tissues of the eye.

The main barriers for ocular drug delivery are

(1) Elimination from lachrymal fluid (pre-corneal barrier): most of the instilled volume is either drained from the conjunctival sac into the nasolacrimal duct or cleared from the precorneal area, resulting in poor bioavailability of drugs.

(2) Corneal barrier: anatomically, the corneal barrier is due mainly to intercellular tight junctions (zonula occludens) which completely surround the superficial epithelial cells, serving as a selective barrier for small molecules and completely prevent the diffusion of macromolecules via the paracellular route. Corneal stroma, instead, is a highly hydrophilic tissue with an open structure that allows the diffusion of hydrophilic drugs up to 500 kDa size, while it is a rate-limiting barrier for most lipophilic drugs. The corneal endothelium is responsible for maintaining normal corneal hydration, and it has been estimated that drugs with molecular dimensions up to about 20 nm can diffuse. The drug transport across the corneal epithelium is essential via paracellular or transcellular routes. The hydrophilic drugs penetrate primarily through the paracellular pathway, while lipophilic drugs prefer the

transcellular route. Lipophilicity, solubility, molecular size, charge, and degree of ionization also affect the cornea's route and rate of penetration. Particulate material in the nanometer range has been reported to follow the endocytic pathway depending on the matrix's optimized lipophilic–hydrophilic properties and reduced particle size.

(3) Non-corneal absorption: topically applied ocular drugs may be absorbed through the bulbar conjunctiva and the underlying sclera into the uveal tract and vitreous humor, which results in drug loss at desired site3.

Glaucoma is a multifactorial optic neuropathy and is the second leading cause of blindness worldwide. A major risk factor is increased IOP in the eye when the ratio between aqueous humor formation (inflow) and its outflow is unbalanced. Lowering IOP via various pharmaceuticals and/or surgical techniques is currently the mainstay of glaucoma treatment. The topical route is the one most commonly used, owing to its suitability for chronic administration. Drug bioavailability can be improved by the delivery system, also decreasing the dosage. Treatment options are 'inflow inhibitors' (beta-antagonists, carbonic anhydrase inhibitors) and 'outflow enhancers' (alpha agonists, prostaglandin analogs, and miotic agents). Innovative 'inflow' (hydroxysteroid dehydrogenase-1 inhibitors; melatonin) and 'outflow' agents (dopamine, serotonin, adenosine agonists, cannabinoids) are currently under study3.

MATERIALS AND METHODS

Brinzolamide, Sodium glycocholate, Polysorbate 80, Boric acid, Mannitol, Disodium EDTA dihydrate Soya phosphotidyl choline, Boric acid, and Purified Water.

Manufacturing Process4-8

PART 1: Organic Phase preparation

1. Measured quantity of 15mL Methanol taken in a clean and dried Rota evaporator flask
2. Dispensed quantities of Soya phosphotidyl choline and Sodium glycocholate were added to

above step 2 and stirred for 25 minutes or until dissolve completely.
3. Dispensed quantity of Polysorbate 80 was added to the above step and stirred for 10 minutes.
4. To the above step required quantity of Brinzolamide was added and stirred for 15 minutes to dissolve completely.
5. Above sample was subjected to Rota evaporation for about 120 minutes and applied a vacuum of about 100mbar and the water bath temperature was maintained at around 60 to 65 o C for product evaporation.
6. After solvent evaporation a thin slightly yellow colour shiny layer formed on the inner walls of the round bottom flask, Rota evaporator flask was removed by releasing the vacuum for further processing, and the water bath temperature was maintained at around 60 to 65.

PART 2: Aqueous phase preparation

7. 50% of the batch size nitrogen purged purified water collected in a cleaned beaker
8. To the above step required quantity of Disodium EDTA dihydrate was added and stirred for 15 minutes
9. To the above step required quantity of Mannitol & Boric acid was added and stirred for 15 minutes or completely dissolved.
10. To the above step adjust the pH to 7.1 ± 0.2 by using 1N sodium hydroxide.
11. The above solution was heated up to 60 to 65°C in a water bath.

PART 3: Mixing of Part 1 & Part 2

12. Hydration of the film is done by gradually adding the above solution from step number 11 to the dried thin film in the Rota evaporator flask from step number 6 and hydration was done for up to 1 hour under continuous stirring.
13. After hydration, volume make up to 100% and subjected to filtration; through a 0.2µ filter, and Physical parameters were performed after filtration.
14. Prepared formulations were subjected to excretion studies for 2 weeks at 40°C and 60°C

TABLE 1. Manufacturing Process

S. No	Ingredients	Manufacturer	Concentration	
			In (%)	In (mg/mL)
1	Brinzolamide	Flax Laboratories	0.5%	5
2	Soya phosphotidyl choline	Lipoid GMBH	0.7%	7.0
3	Sodium glycocholate	Prodotti chemicals	0.3%	3.0
4	Polysorbate 80	Croda Inc	3.0%	30
5	Boric acid	C G Chemikalien	0.10%	1.0
6	Mannitol	Roqutte	2.5 %	25
7	Disodium EDTA dihydrate	C G Chemikalien	0.1%	1.0
8	Sodium hydroxide	Rexler-Planet Science	q.s. to adjust pH 7.0 -7.40	
9	Hydrochloric acid	C G Chemikalien		
10	Purified Water	In-House	q.s. to 1mL	

Key features of container closure system

Preservative-Free Multi Dose eye dropper delivering consistent drops. A one-way valve dispenses the dose and prevents back-flow to maintain the sterility of the bulk. A silicone membrane allows air to enter the container by diffusion while preventing contamination ingress. Air diffuses through the silicone which acts as a semipermeable membrane to air. The liquid remaining on the outside of the one-way valve is protected from bacterial growth by the presence of silver ions in the outer components, silver ions are only in contact with the remaining drop which is prevented from returning to the bottle by the non-return valve. The one-way valve closes preventing liquid from being sucked back into the bulk, thus preventing any retro-contamination by liquid backflow.

The utmost recurrently used preservative, Benzalkonium chloride (BAK) has steadily

proved its toxic effects in laboratory, experimental and clinical studies. Other preservatives used in eye drops are typically chemical preservatives like BAK or, more recently, oxidative preservatives. However, these are still preservatives and have some toxicity. Users find multi dose eyedroppers easy and appropriate to use and advantage from their simplicity rather than the bulk associated with multi-unit dose packs.

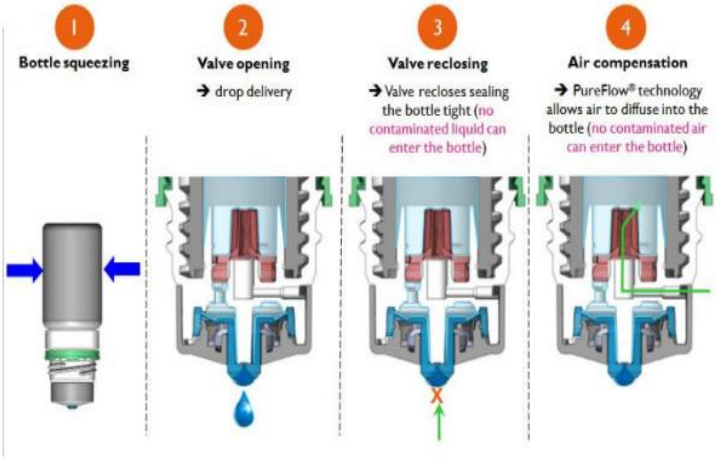

FIG.1. System functioning

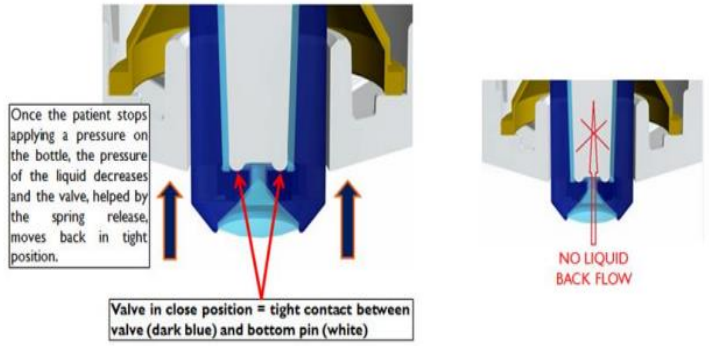

FIG 2. container closure function system

Stability studies15-17

Stability studies performed as per this guideline by loading the samples at different conditions like 25°C ± 2°C not more than 40% RH ± 5% RH for the long term & 40°C ± 2°C Not more than 25% RH accelerated the condition. These stability studies done at these 2 conditions is applicable to all zones, based on the climatic conditions of these countries mean kinetic temperature is derived and applicable to any country in the world, where the countries are divided majorly into four climatic zones. i.e., zone I to zone IV. This guidance mainly describes stability conditions required for climatic zone I & zone II, but the principle established in this guidance applies to other regions of EC, Japan and the United States are also applicable to the other two regions' stability studies performed in accordance with this guideline.

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RESULTS

TABLE 2. Physical Parameters and Observation of trail 4

S. No	Tests	Results
1	Description	Slightly yellowish clear solution
2	D10	11.2 nm
	D50	21 nm
	D90	49.6 nm
	Z- average	19.12 nm
	PDI	0.269
3	pH	6.88
4	Osmolality	305 mOsmol/kg
5	Assay	103.4% (2.585 mg/mL)
6	Free drug	0.493 mg/mL
7	Entrapped drug	2.0920 mg/mL
8	Encapsulation efficiency	83.68%

Evaluation Studies

TABLE 3: Physical Parameter Observation

S. No	Tests	Trail 1	Trail 2	Trail 3
1	Description	Clear solution	Clear solution	Clear solution
2	Osmolality	NA	NA	311 mOsmol/kg
3	pH	7.4	7.2	7.2
4	Drop Size	26.9 µl	29.7 µl	28.8 µl

Optimization Batch study results

TABLE 4: Physical Parameters and Observation

S. No	Tests	Results
1	Description	Slightly yellowish clear solution
2	D10	11.5 nm
	D50	21.8 nm
	D90	49.9 nm
	Z- average	19.78 nm
3	pH	6.88
4	Osmolality	305 mOsmol/kg
5	Assay	101.8%
6	Free drug	0.655 mg/mL
7	Entrapped drug	4.435 mg/mL
8	Encapsulation efficiency	88.7%

In vitro Diffusion Study of Optimised Batch

TABLE 5: In vitro Diffusion Study

S. No	Time in Min	Drug released/ Diffused in (µg/Sq. Cm)	Cumulative drug released/Diffused (µg/Sq. Cm)
1	0	0.0	0.0
2	15	331.2	331.2
3	30	723.9	842.2
4	45	729.3	1106.2
5	60	521.9	1159.2

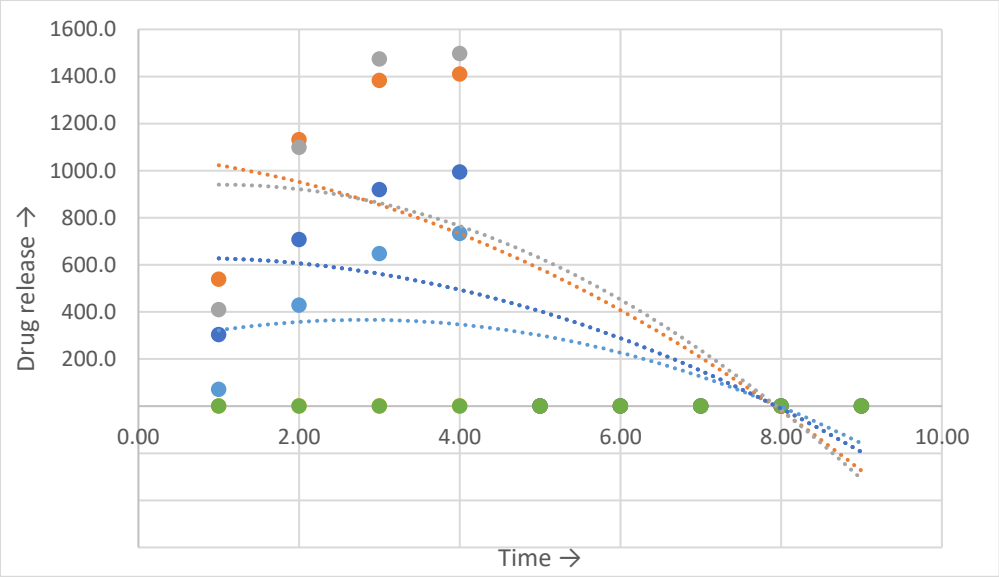

FIG 3. In vitro Diffusion Study

Stability Studies

TABLE 6. ICH guidelines for the stability study

S. No	Parameters		Storage condition			
		25°C ± 2°C/ 40% RH ± 5% RH 3M	25°C ± 2°C/ 40% RH ± 5% RH 6M	45°C / 25% RH, 1 M	45°C / 25% RH, 3 M	45°C / 25% RH, 6 M
1	Particle size (nm)	--	19.01	--	--	24.45
2	Assay (%)	101.2	101.9	101.0	103.9	107.5
3	Osmolality	314	307	301	299	302
4	Zeta potential	--	-2.45 mV	--	--	-1.13 mV

DISCUSSION

The evaporation method successfully prepared BZ NMs for ophthalmic drug delivery. The selected formulations exhibited a good drug release profile with appropriate physicochemical characteristics. Various components in NMs formulation (Trial-4) without preservatives facilitated BZ penetration into the corneal tissue with lower drug concentrations (0.4%) in comparison with the commercial product (1%)

still resulting in higher therapeutic efficacy in-vitro. These findings suggest that NMs could be a promising delivery platform for enhanced BZ delivery.

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